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14. ABSTRACT

This research resulted in the design and synthesis of new nanoconjugates with the ability to target multidrug resistant tumors. Additionally, the work has helped to initiate a new research program focused on the delivery of drugs to the brain. Both tracks of research are continuing in the PI's laboratory. Most surprisingly, we discovered that a simple P-gp substrate that has no other pharmacological effects, is easily conjugated to the delivery system and effectively targets MDR cells. This is particularly exciting because for drug targeting to the brain, having minimal off-target pharmacological activity is important toward the development of an effective delivery vehicle (i.e., low side effects).

15. SUBJECT TERMS
Multidrug resistance, nanoconjugate, targeted drug delivery

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1. Introduction

The premise of the proposed research was to utilize the efflux pump mechanism of P-glycoprotein to target drug-containing particles to multidrug resistant cells. Conceptually, the proposed targeting mechanism is shown in cartoon form in Figure 1. The idea represents a new way of thinking about how to use P-gp as a targeting opportunity. Generally the efflux activity of P-gp is considered a detriment to the treatment of tumors because it actively removes the anticancer agents from the cytosol of the cell. However, using the targeting paradigm shown in Figure 1, the proposed research aims to use P-gp as an opportunity to selectively target and kill cells that express the protein.

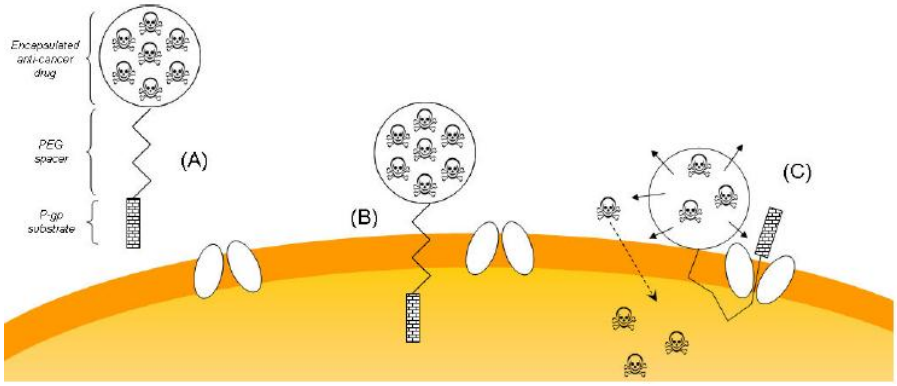

Figure 1. *Tethering of drug delivery carriers to cells through interaction with P-gp.* (A) Drug carrier with PEG linker and P-gp substrate, Rho123. (B) PEG linker spans membrane as Rho123 diffuses into cell. (C) Export of Rho123 by P-gp tethers drug carrier to cell.

The hypothesis for the research, as originally written in the IDEA proposal was that drug targeting to breast cancer can be mediated by cells that overexpress P-gp, and the P-gp anchored drug carrier can improve the therapeutic efficacy of the anticancer drugs. The overall purpose of the grant was to exploit P-gp as a cell-type specific target for the targeted treatment of breast cancer.

The *Specific Aims* of the research were:

1) to design and create drug delivery carriers targeted to cells that overexpress P-glycoprotein

2) to demonstrate P-gp specific binding of drug delivery carriers to breast cancer cells *in vitro*

Each Specific Aim was successfully completed, as shown in the research accomplishments section below.

2. Body

Specific Aim 1: *to design and create drug delivery carriers targeted to cells that overexpress P-glycoprotein*

The synthesis of PEG tethered to the P-gp substrate, rhodamine123 was conducted as shown in Figure 2. Confirmation of the conjugate structure was determined by ^{1}H NMR (Figure 3)

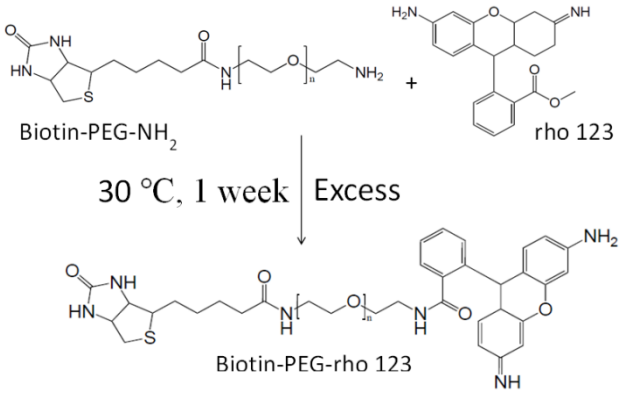

Figure 2. Conjugation reaction of biotin-PEG-NH_2 to the P-gp substrate, rhodamine 123. All reactions were carried out in dimethylformamide (DMF) in the presence of excess triethylamine. A 3:1 mol ratio of rho 123:NH_2 was used. Purification was conducted by dialysis against distilled water, and final chemical structure determined by ^{1}H NMR

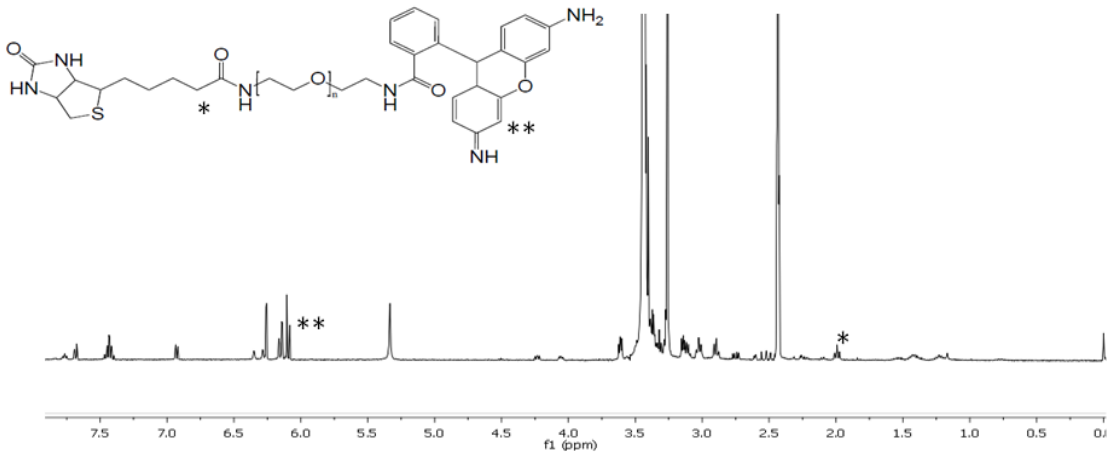

Figure 3. ^{1}H NMR showing 94% conjugation. (*) the peak from biotin (**) peak from rhodamine 123, integrated peak area used to calculate percent conjugation

The model delivery system consisted of the biotin-PEG-Rho123 conjugate attached via the avidin-biotin link to fluorescently labeled avidin-coated polystyrene beads. The beads allow the visualization of the system by fluorescent microscopy thereby allowing the quantitation of binding in the follow-on flow chamber experiments (Specific Aim 2). A cartoon representation of the final conjugate is provided in Figure 4.

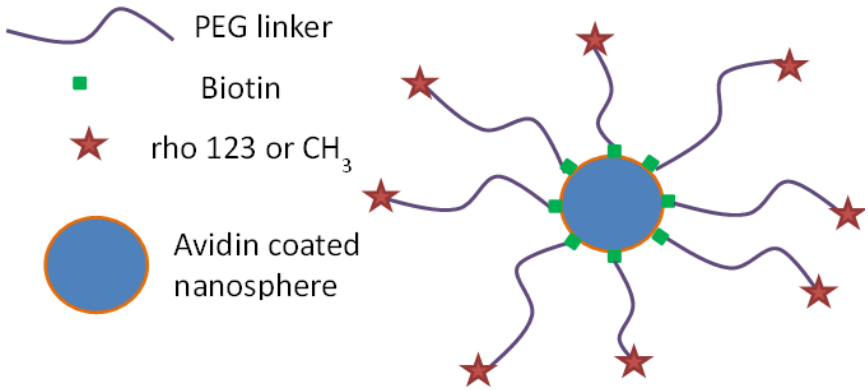

Figure 4. Fluorescent polystyrene nanoparticle used for initial targeting studies. Targeted form includes a rho123 end group. Untargeted control contains a CH_3 end group.

Specific Aim 2: *to demonstrate P-gp specific binding of drug delivery carriers to breast cancer cells in vitro*

Flow chamber experiments were conducted to determine the binding capacity of the P-gp particles to P-gp expressing breast cancer cells compared to non-P-gp expressing breast cancer cells. The configuration of the flow chamber is provided in Figure 5.

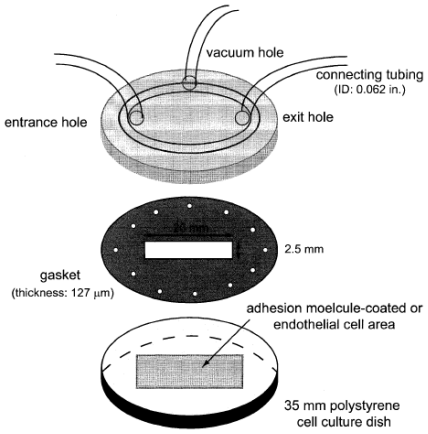

Figure 5: Schematic diagram of a circular parallel-plate flow chamber. Cells are grown on a glass surface over which a gasket is placed allowing the flow of reagents over the top of the cells. Attachment of beads to the cell surface is quantified by use of a fluorescent microscope positioned above the flow chamber visible window.

The MCF-7 breast adenocarcinoma cell line, engineered to either express P-gp (MCF-7/Mdr1) or not express P-gp (MCF-7/wt) were grown on the surface of a glass plate. Expression or absence of P-gp in each cell line was determined by Western blot analysis (Figure 6).

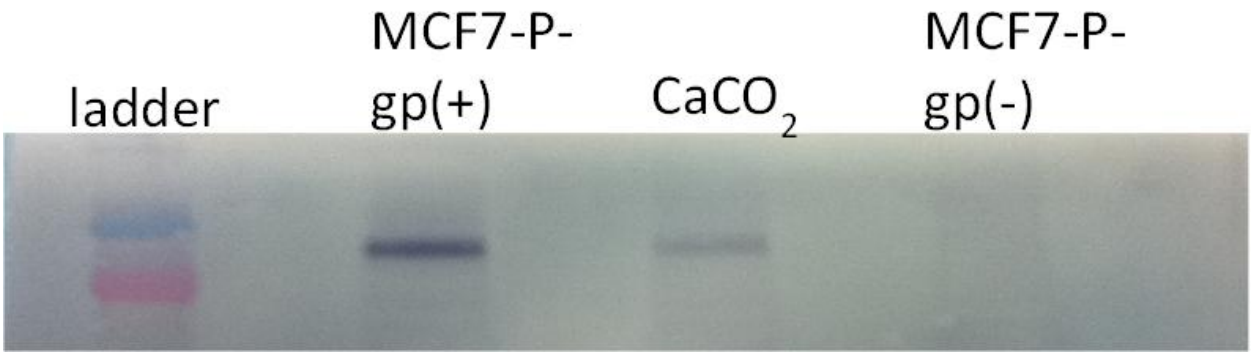

Figure 6: Western blot showing P-gp over expression in MCF7-P-gp(+) cells compared to positive control CACO-2 cells and negative control MCF7-P-gp(-) cells.

FACS analysis was used to quantify the number of targeted and untargeted beads to both P-gp expressing and P-gp non-expression cells (Figure 7).

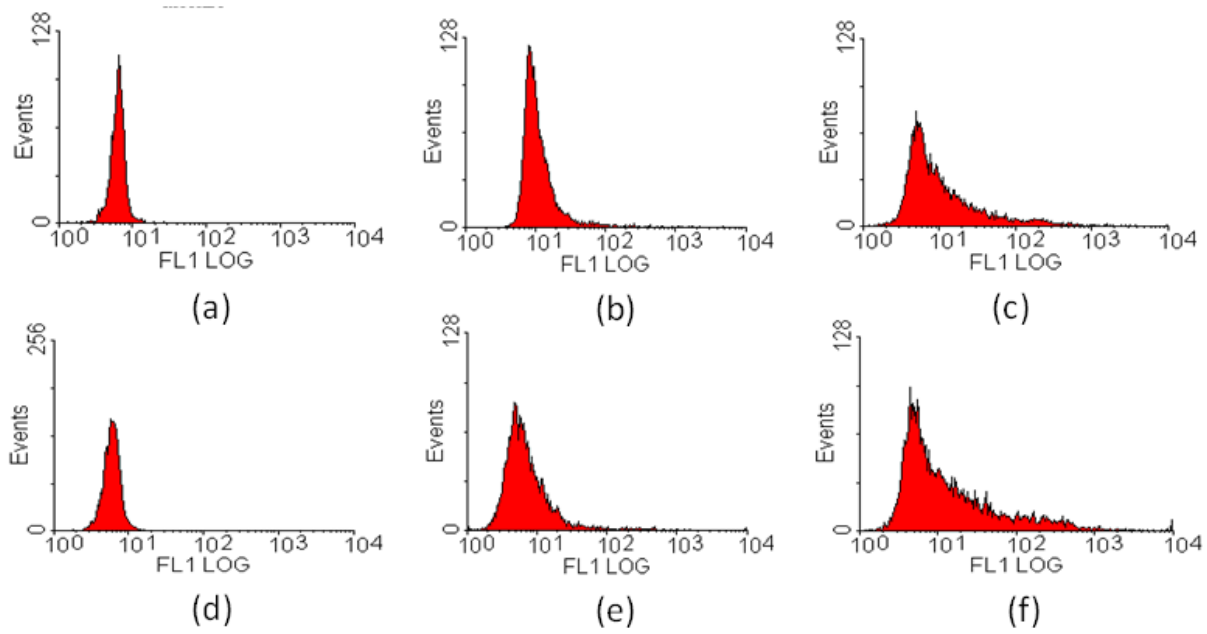

Figure 7: FACS data in initial in vitro targeting studies. (a) MCF7-P-gp(-) cells (b) MCF7-P-gp(-) cells with control beads (c) MCF7-P-gp(-) cells with rho 123 beads (d) MCF7-P-gp(+) cells (e) MCF7-P-gp(+) cells with control beads (f) MCF7-P-gp(+) cells with rho 123 beads

From the flow chamber data, the total number of bound beads were quantified (experiments conducted in triplicate). The output from the experiment is shown in Figure 8 below.

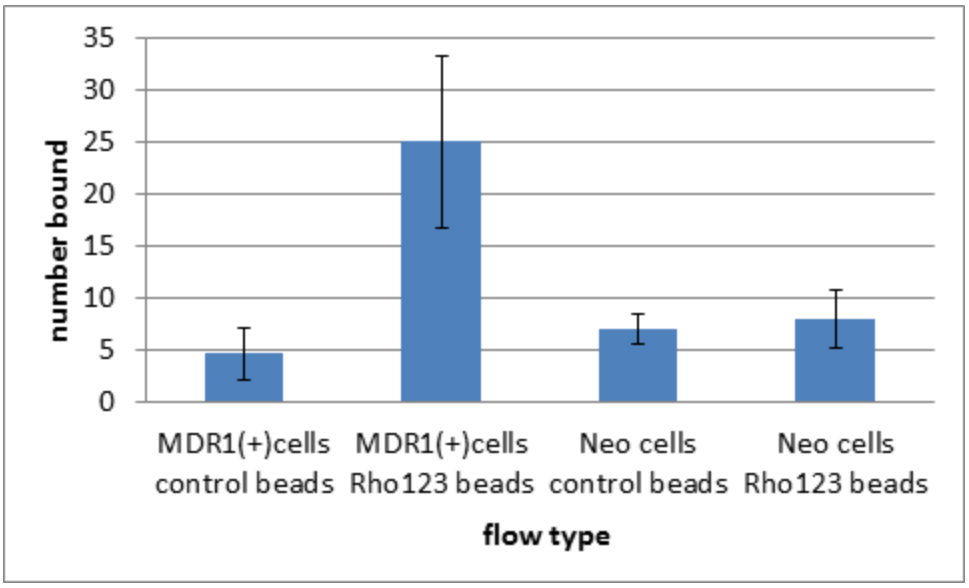

Figure 8: Flow chamber data from flow chamber studies. The results show that control beads (without rhodamine) do not adhere to either P-gp expressing or P-gp expressing MCF-7 cells, whereas beads containing rhodamine123 adhere to P-gp expressing MCF-7 cells, but not to non-expressing cells. Collectively these data demonstrate the feasibility of using P-gp substrates to target MDR(+) tumors.

Lastly, the kinetics of rhodamine binding to MDR(+) cells (i.e., MCF-7/Mdr1) was determined over a 30 min timeframe (Figure 9). The data reveal a rapid binding curve followed by an approximately first-order elimination followed by a steady state concentration. These data suggest a potential validation of the "fishing hook" hypothesis for how these conjugates bind to P-gp(+) cell lines.

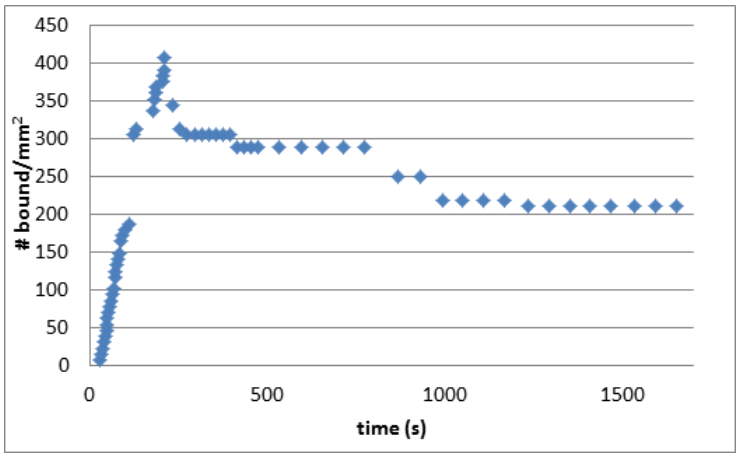

Figure 9: Binding kinetics profile of rhodamine-targeted beads to MCF-7/Mdr1 cells.

3. Key research accomplishments

Each proposed Specific Aim was successfully completed. The conjugation of a P-gp substrate to PEG-biotin allowed the formulation of polystyrene beads with the ability to selectively bind to P-gp(+) cells. The degree of binding was successfully quantified using a flow chamber mounted to a fluorescent microscope and FACS. Collectively, these data suggest that a P-gp substrate can be successfully used to selectively target P-gp(+) cell types.

4. Reportable outcomes

The output from these successful experiments demonstrate the feasibility to selectively target drug-conjugates to P-gp(+) cell types. This is the first demonstration of the utilization of P-gp as a targeting receptor.

5. Conclusions

The data resulting from the two Specific Aims strongly support, for the first time, the validity of utilizing P-gp substrates as agents for targeting P-gp(+) cell types.

6. References

None required for this work. This is the first example of using P-gp substrates as a targeting agent.

7. Appendices

None.